Bull. Org. mond. Santé }
Bull. Wld Hlth Org. } 1973, **48**, 415-420

Penicillamine as an adjuvant to antimonial therapy of schistosomiasis: effect on liver function tests in rabbits and on antischistosomal activity *

M. T. KHAYYAL, S. SALEH, & A. M. EL MASRI

Earlier work has shown that penicillamine reduces the acute toxicity of antimonyl potassium tartrate (APT) as well as the abnormal ECG changes it induces. In the present study, the possible protective effect of penicillamine on the hepatic toxicity of APT was investigated. Tests of liver function showed changes in the level of serum aspartate and alanine aminotransferase and of alkaline phosphatase, and in the beta-/alpha-lipoprotein ratio, in response to antimony treatment. The changes were significantly reduced by penicillamine, though the effect depended on the dose. Penicillamine was found to give the best overall protection without affecting the antischistosomal efficacy of the antimonial when a 1 : 2 APT/penicillamine ratio was used. The findings provide further evidence of the potential usefulness of penicillamine in the antimonial treatment of schistosomiasis.

The high toxicity of antimonials has limited their use in the treatment of human schistosomiasis. This toxicity may be a consequence of inhibition of —SH enzymes in the cell, an effect that can probably be antagonized by mercapto compounds. Among the many reports on the experimental use of mono- and dimercapto compounds to combat heavy metal poisoning, those concerning penicillamine (3-mercaptovaline) have been studied extensively in recent years. Penicillamine was found to reduce markedly the acute toxicity of potassium bis[μ-tartrato(4-) diantimonate(2-)] dihydrate (antimonyl potassium tartrate—APT) in hamsters and mice (Ercoli, 1967; Khayyal et al., 1967). The cure rate was not affected by the addition of penicillamine to treatment with APT (Khayyal et al., 1967) at certain dose levels. Moreover, the incidence of abnormal ECG changes characteristic of antimonial therapy was greatly reduced (Girgis et al., 1970).

The present study was designed to determine if penicillamine would reduce the undesirable effect of APT therapy on liver function in rabbits and to show the effect of combined therapy on the mortality rate. Several proportions of penicillamine to APT were investigated to find the ratio that most reduced the hepatotoxic effects of the antimonial without affecting its antischistosomal activity. The antischistosomal activity of the various combinations was studied both *in vitro* and *in vivo*.

* From the Department of Pharmacology, Faculty of Pharmacy, Cairo University, Cairo, Egypt.

MATERIALS AND METHODS

Liver function

Five biochemical tests were chosen to evaluate liver function. The serum aspartate aminotransferase (2.6.1.1) and alanine aminotransferase (2.6.1.2) were determined by the method of Reitman & Frankel (1957). The serum alkaline phosphatase (3.1.3.1) was determined by the method of King et al. (1942). The thymol turbidity and flocculation were tested by the method of Shank & Hoagland (1946), and the icterus index was measured. Lastly, the serum lipoproteins were separated by electrophoresis as described by Swahn (1952), and the ratio of beta- to alpha-lipoproteins was established.

A group of 10–13 male rabbits was used for each of the dose regimens applied. Liver function tests were performed before treatment was started. One group received penicillamine in a dose of 8 mg per kg of body weight. Other groups were given APT in a dose of 4 mg/kg either alone or in combination with penicillamine in ratios of 1:1, 1:2, 1:3, and 1:4. The drugs were given intraperitoneally for 5 consecutive days. Samples of blood for liver function determinations were drawn from the animals by heart puncture

24 hours and 2 weeks after the last injection. The animals were fasted for 24 hours before blood samples were taken. Because of the influence of diet on the liver and its functions, the animals were kept on a standard diet of clover and bread for 2 weeks before the experiment began.

For each dose regimen, an untreated control group was maintained in the laboratory.

Antischistosomal activity

In vitro activity was studied by incubating *Schistosoma mansoni* worms recovered from infected animals in a medium consisting of equal parts of horse serum and Tyrode's solution (Standen, 1962). Two pairs of worms were incubated at 37°C in 5 ml of medium in each of a series of sterile tubes. Of 7 duplicate sets of tubes, a control set contained no drug while the other 6 sets contained APT at a concentration of 0.02% (equivalent to 5.9×10^{-4}M) alone or in combination with penicillamine at concentrations of 0.02%, 0.04%, 0.06%, 0.08%, and 0.10%, respectively. This corresponds to ratios of 1:1, 1:2, 1:3, 1:4, and 1:5 by volume, or approximately 1:2.2, 1:4.5, 1:6.7, 1:9.00, and 1:11.2 in terms of molarity. All the sets were placed in the incubator and examined every half hour for signs of paralysis. The time of onset of paralysis for each tube was taken as the average time to immobilize all the parasites in the tube.

In vivo activity was considered from two aspects: the early hepatic shift and the cure rate. To study the early hepatic shift, 4 batches of 30 mice were used. The mice had each been infected with 150 cercariae of an Egyptian strain of *S. mansoni* 7 weeks before the experiment. One batch of mice was injected intraperitoneally with 20 mg of APT per kg of body weight while the other 3 batches were given the same dose of APT combined with penicillamine in doses of 20 mg/kg, 40 mg/kg, and 60 mg/kg respectively. The penicillamine was given by separate injection immediately following the administration of APT. Each batch was subdivided into 3 groups of 10 animals, which were autopsied at 2, 4, and 24 hours after injection. The number of worms, their sex, and their location in the hepatic portal circulation were carefully observed and recorded. For each batch of infected mice, an untreated control group was maintained.

To study cure rates, 4 batches of 10 infected mice each were used, in addition to a control batch. The batches were given intraperitoneal injections of APT alone or in conjunction with penicillamine in ratios of 1:1, 1:2, and 1:3. The injections were given daily for 5 days. Two weeks after the last injection, the animals were autopsied and the distribution of schistosomes in the hepatic portal system of each mouse was recorded.

RESULTS

Effect of combined treatment on mortality

The mortality in the group of rabbits receiving APT alone was found to be 40% 24 hours after the last injection but increased within 2 weeks to 80%. In contrast, 2 weeks after combined therapy with APT and penicillamine at a ratio of 1:2 that mortality rate was nearly halved. This was the best protection afforded by the adjuvant regimens tested.

Effect on the liver functions

The icterus index and thymol turbidity tests showed only minor and insignificant differences from the normal throughout the experimental period with all regimens tested and have therefore been omitted from the results illustrated. The effect of APT and pcnicillamine alone or combined in various ratios on the serum enzyme levels and on the beta-/alpha-lipoprotein ratio is shown in Fig. 1.

When APT was used alone in a dose of 4 mg/kg for 5 consecutive days, it was observed that 24 hours after the last injection there was a 3-fold increase in both aspartate and alanine aminotransferase values compared to untreated controls, a 2-fold increase in alkaline phosphatase, and more than a 10-fold increase in the beta-/alpha-lipoprotein ratio. There was still a significant difference in the values 2 weeks after treatment. Penicillamine alone, administered in a dose of 8 mg/kg, caused an abrupt but short-lived rise in aspartate aminotransferase and a smaller but more sustained rise in alanine aminotransferase; the alkaline phosphatase value was halved after 24 hours but returned to normal within 2 weeks. The beta-/alpha-lipoprotein ratio showed a transient increase after 24 hours.

A comparison of the various combinations of APT and penicillamine indicated that the extent of protection was dependent on the amount of adjuvant used. Thus, as the proportion of penicillamine increased, there was a corresponding increase in protection against the aspartate aminotransferase changes induced by APT alone, reaching more or less complete protection at a ratio of 1:4. The smallest deviations from the normal values for alanine aminotransferase were observed when

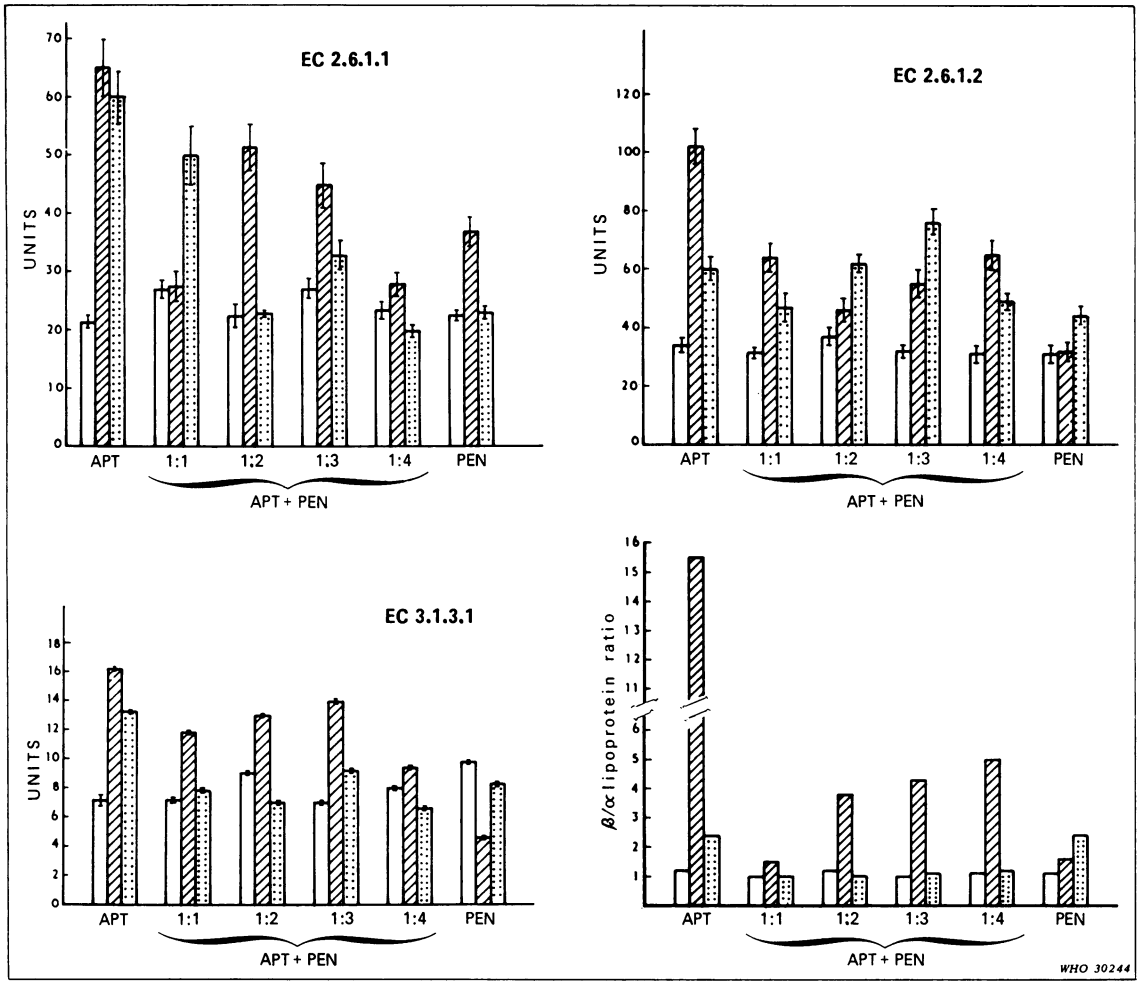

Fig. 1. Effect of treatment with APT and penicillamine (PEN) alone or combined in various ratios on serum enzyme and beta-/alpha-lipoprotein levels of rabbits treated daily for 5 days. Blank columns, normal; shaded columns, 24 h after last injection; stippled columns, 2 weeks after last injection. The histograms show mean values, with standard errors for serum enzyme levels.

using ratios of 1:1 and 1:2. The alkaline phosphatase values were initially raised with all combined regimens tested, although to significantly lower levels than following treatment with APT alone. Two weeks after treatment, that effect was reversed; alkaline phosphatase activity decreased significantly, especially with the 1:2 and 1:4 ratios.

As to the lipidogram, the best protection against the changes provoked by APT alone was observed in the 1:1 combination, which caused only minimal changes from the normal in the beta-/alpha-lipoprotein ratio. Higher proportions of the adjuvant gave less protection and the beta-/alpha-lipoprotein ratio rose, though not to the same extent as with APT alone.

Effect on schistosomes

Table 1 shows the antischistosomal activity of the various drug combinations *in vitro*. As the concentration of adjuvant in the medium was increased from 1 to 5 times the concentration of APT there was a corresponding delay before the schistosomes became paralysed; the delay ranged from 20% with the 1:1 and 1:2 ratios to 80%, 100%, and 180%

Table 1. *In vitro* incubation of 2 pairs of adult *S. mansoni* with APT alone or in combination with penicillamine in various ratios

Drug concentration in medium		Time of observation after exposure (hours)[a]									
APT	Penicillamine	½	1	1 ½	2	2 ½	3	3 ½	4	4 ½	5
control	—	4	4	4	4	4	4	4	4	4	4
0.02 %	—	4	2	1	—	—	—	—	—	—	—
0.02 %	0.02 %	4	3	2	1	—	—	—	—	—	—
0.02 %	0.04 %	4	3	2	1	—	—	—	—	—	—
0.02 %	0.06 %	4	4	4	1	1	—	—	—	—	—
0.02 %	0.08 %	4	4	4	3	3	2	—	—	—	—
0.02 %	0.10 %	4	4	4	4	4	3	2	1	—	—

[a] Figures in table show number of mobile worms.

with the 1:3, 1:4, and 1:5 ratios. The control worms in drug-free media survived up to 4 days without change of medium.

The *in vivo* experiments with mice showed that as the ratio of penicillamine to APT was increased, the magnitude as well as the duration of the early hepatic shift induced by a single injection was progressively affected. The greater the proportion of adjuvant, the faster was the return of parasites to the mesenteric veins. A progressive reduction in cure rates was also observed as the proportion of penicillamine increased (Table 2). There was no significant reduction in cure rates with a 1:1 APT/penicillamine ratio, a 13% reduction with a 1:2 ratio, and a marked decrease of 75% with a 1:3 ratio.

DISCUSSION

Following antimonial therapy, there was a significant increase in serum aminotransferase levels. Similar increases have been reported by various workers (Tribouley & Duret, 1963; Abdalla et al., 1964; Asshauer, 1966; Lambert, 1966; Coutinho & Barreto, 1969). Melby et al. (1959) and Khattab et al. (1967) postulated that the increase in serum aminotransferases found in the liver cells may denote abnormal permeability of the cell membrane, so that the enzymes are liberated into the blood without necrosis of the cell.

Experiments to date have not provided a full explanation of the toxic reactions of the host to

Table 2. Comparative cure rates after treating mice infected with *S. mansoni* with 5 daily injections of APT alone or in conjunction with penicillamine in various ratios [a]

Treatment	Daily dose (mg per kg body weight)	No. of mice in group	Mean worm burden	% distribution of worms [b]	
				Mesentery	Liver
Untreated control	—	10	22.22 ± 3.40	95.33 ± 2.05	4.67 ± 2.05
APT	20	10	18.37 ± 2.12	7.99 ± 3.79	92.01 ± 3.79
APT + penicillamine	20 + 20	10	19.17 ± 0.85	8.98 ± 4.04	91.02 ± 4.04
APT + penicillamine	20 + 40	10	17.00 ± 1.50	19.47 ± 6.13	80.53 ± 6.13
APT + penicillamine	20 + 60	10	20.33 ± 2.32	77.21 ± 4.16	22.79 ± 4.16

[a] All results are given as mean ± standard error.

[b] Mice were autopsied 2 weeks after completion of treatment. Worms then in the liver were either ensheathed or dead.

antimony. However, most of the evidence seems to suggest that the toxic action may be due to an intracellular combination of antimony with the —SH groups necessary for the catalytic and metabolic activities of tissue cells, leading to inactivation of the enzyme systems involved in cellular oxidation and other vital processes.

In the present study the changes in aminotransferase level produced during antimonial therapy were markedly reduced by penicillamine. The explanation of this protection may be that the antimony was trapped in a chelated form, so that the —SH enzymes were free to carry on their functions.

The reduction in toxicity cannot be attributed to an increase in the excretion of the compound or a decrease in tissue uptake, especially with high ratios of APT to penicillamine. Khayyal et al. (1967), using APT and penicillamine in a ratio of nearly 1:2, were unable to demonstrate any reduction in tissue antimony level after 24 hours. Other workers found that when this ratio was reduced to 1:5 (Tarrant et al., 1971) there was a marked increase in the rates of both urinary and faecal excretion of the antimonial; however, they did not attempt to use higher ratios in their elimination studies. Penicillamine's protective effect against mortality from APT, in the high ratios at least, may therefore be due to the conversion of APT to a less toxic, possibly chelated form in the tissues, and not to enhanced excretion, which only occurs with low APT/penicillamine ratios.

APT alone produced a moderate but significant increase of about 120% in serum alkaline phosphatase. A rise in this enzyme without overt jaundice has recently been linked with liver parenchymal disease (Hill & Sammons, 1967). Since there was no rise in icterus index in the animals tested, jaundice was ruled out; the rise in alkaline phosphatase most probably reflects liver parenchymal damage.

Penicillamine was found to safeguard against an increase in serum alkaline phosphatase. Indeed, the level even fell below normal after administration of penicillamine alone. It is possible that penicillamine, as it is capable of chelating many of the essential ions (Lenz & Martell, 1964), may deprive the body of Mg^{2+}, known to be a possible activator for alkaline phosphatase (Erdtman, 1928). The ability of penicillamine to inhibit this enzyme may thus be responsible, at least in part, for its protective effect against the rise of the serum enzyme level in rabbits treated with a combination of APT and penicillamine.

APT therapy was shown to increase the beta-lipoprotein and decrease the alpha-lipoprotein, so that the beta-/alpha-lipoprotein ratio rose. Penicillamine gave the best protection at the 1:1 ratio.

Taken as a whole, the studies on the host indicated that APT alone was rather toxic to the liver. The ratio of penicillamine to APT that afforded best protection varied from test to test, pointing to the need to select the best overall prophylactic/adjuvant ratio provided that it would not appreciably reduce the antischistosomal effect. The cure rate studies in infected mice, as well as the effect of the various ratios on the worms *in vitro*, showed that the 1:1 and 1:2 APT/penicillamine combinations had the least effect on the activity of the antimonial. Bueding & Fisher (1966) have shown that the inhibition of worm phosphofructokinase (2.7.1.11) by antimonials is not affected by mercapto compounds, indicating that this inhibition is not brought about by interaction of APT with the mercapto groups of schistosome phosphofructokinase. On the other hand, if the antimonial's toxicity to the host is really based on inhibition of mercapto enzymes in the body, penicillamine would be expected to reduce the toxicity to the host more than to the parasites. This is what has been observed, and confirms the findings of Khayyal et al. (1967). High ratios of penicillamine similar to those used in the experiments of Tarrant et al. (1971)—penicillamine in a dose of 100 mg/kg 3 times daily with a single 20 mg/kg dose of APT—were found to affect appreciably the antischistosomal activity of the antimonial both *in vivo* and *in vitro*. It should be stressed that it is the ratio of penicillamine used that is the decisive factor in determining its effect on the antischistosomal and hepatotoxic activity of APT. Tarrant et al. (1971) did not attempt this treatment approach.

The exact mechanism of the protective effect of penicillamine is not well understood. Chelation of antimony is a likely possibility, but whatever the explanation, it seems that an APT/penicillamine ratio of 1:2 is optimal. In support of that conclusion, Girgis et al. (1970) have found that the same ratio protected dogs against APT-induced ECG changes. Thus the two organs mainly affected by antimonial toxicity—the liver and heart—are afforded protection through the concomitant use of penicillamine. Recently, Ercoli (1971) compared APT (and other antimonials) with a chelate of the corresponding sodium compound and penicillamine and found a higher chemotherapeutic index for the chelate. Moreover, the chelate has been used intramuscularly

in patients, thus offering an advantage over APT. Further work is now being done clinically on a limited scale to investigate the therapeutic usefulness of combining penicillamine with APT therapy in view of the beneficial effects of the adjuvant that have been observed experimentally.

RÉSUMÉ

LA PÉNICILLAMINE EN TANT QU'ADJUVANT DU TRAITEMENT DE LA SCHISTOSOMIASE PAR LES ANTIMONIÉS: ACTION SUR LES TESTS DE LA FONCTION HÉPATIQUE CHEZ LE LAPIN ET SUR L'ACTIVITÉ ANTISCHISTOSOMIENNE

On a étudié chez le lapin les modifications des fonctions hépatiques après traitement par le tartrate d'antimoine et de potassium (TAP) seul ou associé dans des proportions variables à la pénicillamine.

L'administration du composé seul entraîne une élévation des taux des aminotransférases sériques et de la phosphatase alcaline et une augmentation du rapport bêtalipoprotéine/alphalipoprotéine. L'injection simultanée de pénicillamine confère une protection contre ces troubles dans une mesure variant suivant les proportions utilisées. L'effet de la pénicillamine, à diverses doses, sur l'activité antischistosomienne du TAP a été aussi étudiée sur *Schistosoma mansoni in vitro*, et *in vivo* chez des souris infectées.

Si l'on compare l'action de la pénicillamine, en fonction des proportions utilisées, sur l'activité antischistosomienne du TAP et sur son hépatotoxicité, on constate que l'association du TAP et de la pénicillamine dans le rapport de 1: 2 est celle qui assure la meilleure protection des fonctions hépatiques sans altérer sensiblement l'efficacité schistosomicide de la préparation stibiée.

Les auteurs discutent l'intérêt de ces observations en ce qui concerne l'utilisation éventuelle de la pénicillamine comme adjuvant du traitement de la schistosomiase par les antimoniés.

REFERENCES

Abdalla, A. et al. (1964) *J. Egypt. med. Ass.*, **47**, 52

Asshauer, E. (1966) *Arzneimittel-Forsch.*, **16**, 1546

Bueding, E. & Fisher, J. (1966) *Biochem. Pharmacol.*, **15**, 1197

Coutinho, A. & Barreto, F. T. (1969) *Ann. N.Y. Acad. Sci.*, **160**, 612

Ercoli, N. (1967) *Nature (Lond.)*, **216**, 398

Ercoli, N. (1971) *Bull. Wld Hlth Org.*, **45**, 371

Erdtman, H. (1928) *Hoppe-Seylers Z. physiol. Chem.*, **177**, 211

Girgis, N. I. et al. (1970) *E. Afr. med. J.*, **47**, 1

Hill, P. G. & Sammons, H. G. (1967) *J. clin. Path.*, **20**, 654

Khattab, M. et al. (1967) *J. Egypt. med. Ass.*, **50**, 253

Khayyal, M. T. et al. (1967) *Bull. Wld Hlth Org.*, **37**, 387

King, E. J. et al. (1942) *Lancet*, **1**, 207

Lambert, C. R. (1966) *Acta trop. (Basel)*, **23**, 1

Lenz, G. R. & Martell, A. E. (1964) *Biochemistry (Wash.)*, **3**, 745

Melby, J. C. et al. (1959) *Lancet*, **1**, 441

Reitman, S. & Frankel, S. (1957) *Amer. J. clin. Path.*, **28**, 56

Shank, R. E. & Hoagland, C. W. (1946) *J. biol. Chem.*, **162**, 133

Standen, O. D. (1962) In: Wolstenholme, G. E. W. & O'Connor, M., ed., *Bilharziasis*, London, Churchill, pp. 266-286 (Ciba Foundation Symposium)

Swahn, B. (1952) *Scand. J. clin. Lab. Invest.*, **41**, 98

Tarrant, M. E. et al. (1971) *Ann. trop. Med. Parasit.*, **65**, 233

Tribouley, J. & Duret, J. (1963) *Bull. Soc. Pathol. exot.*, **56**, 992